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Evolution and systematics of Green Bush-crickets (Orthoptera: Tettigoniidae: *Tettigonia*) in the Western Palaearctic: testing concordance between molecular, acoustic, and morphological data

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Abstract The genus *Tettigonia* includes 26 species distributed in the Palaearctic region. Though the Green Bush-crickets are widespread in Europe and common in a variety of habitats throughout the Palaearctic ecozone, the genus is still in need of scientific attention due to the presence of a multitude of poorly explored taxa. In the present study, we sought to clarify the evolutionary relationships of Green Bush-crickets and the composition of taxa occurring in the Western Palaearctic. Based on populations from 24 disjunct localities, the phylogeny of the group was estimated using sequences of the cytochrome oxidase subunit I (COI) and the internal transcribed spacers 1 and 2 (ITS1 and ITS2). Morphological and acoustic variation documented for the examined populations and taxa was interpreted in the context of phylogenetic relationships inferred from our genetic analyses. The trees generated in the present study supported the existence of three main lineages: "A"—composed of all sampled populations of *Tettigonia viridissima* and the *Tettigonia vaucheriana* complex, "B"—comprising *Tettigonia caudata*, *Tettigonia uvarovi*, and the *Tettigonia armeniaca* complex, and "C"—consisting of *Tettigonia cantans.* The present study provides the first phylogenetic foundation for reviewing the systematics of *Tettigonia* (currently classified mostly according to morphological characteristics), proposing seven new synonymies.

Keywords *Tettigonia* · mtDNA · rDNA · Phylogeny · Bioacoustics

Introduction

Genus *Tettigonia* Linnaeus, 1758 presently includes 26 recognized species (Eades et al. 2016) distributed in the Palaearctic ecozone and belongs to the long-horned orthopterans or the bush-crickets (Ensifera, Tettigonioidea). *Tettigonia*, popularly known as the Green Bush-crickets, are generally large green orthopterans with moderately slender body and legs and well-developed wings that inhabit the plant cover searching for their food (usually smaller insects or plant tissues). *Tettigonia* is one of the most notable Old World example with two centers of diversity: one in the Mediterranean–Pontic region (see, e.g., Ramme 1951; Pinedo 1985; Chobanov et al. 2014) and another in the Japanese archipelago (see Ichikawa et al. 2006; Kim et al. 2016). Both regions are characterized by a similar number of endemic taxa and insufficient knowledge regarding the taxonomy and systematics of Green Bush-crickets (Ichikawa et al. 2006; Chobanov et al. 2014; own unpublished data).

Despite the fact that several species of Green Bush-crickets are quite well known and have been the subject of detailed neuro-ethological studies (e.g., Zhantiev and Korsunovskaya 1978; Schul 1998), others remain poorly known from single specimens, and even nowadays, the discovery of new species continues (Ogawa 2003; Ichikawa et al. 2006; Chobanov et al. 2014; Storozhenko et al. 2015). Data on the systematics of this genus involve piecemeal morpho-acoustic studies conducted for geographically restricted areas or focused on

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morphological groups of species (e.g., Heller 1988; Rhee 2013; Chobanov et al. 2014). Our recent morphological and acoustic studies on *Tettigonia*, concentrated on the Western Palaearctic, revealed a number of conflicts within the published data when trying to identify certain populations and develop hypotheses about the systematics of the group (own unpublished data). The latter further supports the need to use new markers to test the systematic position of some taxa and unravel the evolutionary history of this genus.

The evolution of acoustic communication systems in orthopterans has led to high levels of acoustic specialization. As acoustic signals are important for intraspecific and sex recognition as well as for interspecific isolation (Paterson 1985; Hochkirch and Lemke 2011), they are of great significance for studying the processes underlying evolutionary radiation. Acoustic diversity within bush-cricket genera varies from very low with a more or less uniform pattern of the male calling song (cf. Heller 2006; Çıplak et al. 2009) to very high with a great variety of song types, especially in sympatric taxa, even within groups of closely related species (cf. Heller 1988, 1990, 2006; Chobanov and Heller 2010, etc.).

In *Tettigonia*, song differences between species (especially well expressed in sympatric taxa) may express in different syllable arrangement and repetition rate, echeme length, and duty cycle. Some differences are also found in the carrier frequency of the song. In some species (e.g., *Tettigonia cantans*), females are not very sensitive to the conspecific structure of the song and thus may respond to heterospecific males (Schul et al. 1998), while in other species (i.e., *Tettigonia caudata*), females rely on the minimum duty cycle of the echemes, thus neglecting the fine song structure (Schul 1998). In *Tettigonia viridissima*, song recognition based on temporal clues has been shown to be more complicated. Here, females evaluate the pause within disyllabic echemes and respond only to the species-specific echeme structure (Schul 1998).

In the present study, we aim to evaluate phylogenetic relationships within *Tettigonia*. We based our study on a genetic dataset that was used as a basis for mapping acoustic and morphological characters in an attempt to track the evolutionary paths of the acoustic communication in this genus. For these purposes, sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene and the nuclear internal transcribed spacers 1 and 2 (ITS1 and ITS2) were used that have previously been widely employed in phylogenetic studies of grasshoppers (e.g., Cooper et al. 1995; Chapco and Litzenberger 2002) and bush-crickets (Ullrich et al. 2010; Allegrucci et al. 2011; Boztepe et al. 2013; Çiplak et al. 2015). The DNA sequences selected for the present study have different modes of evolution and inheritance history, and thus, they may reveal different aspects of the speciation history of the examined lineages.

In bush-crickets, the songs of closely related species, especially those that speciated in allopatry, usually have a lineage-specific amplitude–temporal pattern that enables recognition for systematic purposes and for drawing conclusions about paths of speciation (e.g., Heller 1990, 2006; Chobanov and Heller 2010). In *Tettigonia*, differences between species have been observed in the time and frequency domains, while particular song-recognizing mechanisms may depend on the geographic and ecological preferences of the species (Schul 1994; Schul et al. 1998). Hence, we use the male calling song as an additional clue for evolutionary assumptions as well as for testing the variation of song types according to genetic or morphological units. Thus, the present study indirectly vindicates the significance of acoustic recognition systems and song specialization patterns in this genus.

Material and methods

Taxon sampling and morphological identification

The species used in this study and their sampling localities are presented in Table 1 and Fig. 1. This dataset contains 66 *Tettigonia* specimens from 33 disjunct localities/populations and five outgroup taxa representing two tettigoniid subfamilies (Tettigoniinae: *Amphiestris* Fieber, 1853 (Tettigoniini), *Onconotus* Fischer von Waldheim, 1839, *Paratlanticus* Ramme, 1939, *Platycleis* Fieber, 1853; Saginae: *Saga pedo* (Pallas, 1771)). Due to the properties of the chosen DNA fragments (high amount of interspecific variation providing good phylogenetic signal at a generic level but some risk of false results at a higher systematic level due to convergencies and phenomena like long branch attraction), we choose taxa that, according to published data, are closely related and/or have close ancestral position to *Tettigonia* (in the case of Saginae) (e.g., Gorochov 1995; Song et al. 2015).

Used samples of *Tettigonia* have preliminary been identified using original descriptions and published reviews (e.g., Bolívar 1914; Chopard 1943; Ramme 1951; Harz 1969; Massa 1998; Chobanov et al. 2014 and references therein). All specimens listed in Table 1 were morphologically related to existing taxa based on available literature and museum specimens. Apart from own material, the following specimens from public collections that refer to the studied taxa were studied:

Tettigonia acutipennis Ebner, 1946—male, holotype, “Kleinasien 1914 | Marasch, Tölg. | coll. R. Ebner” (Naturhistorisches Museum Wien (NHMW)); male, Hakkari (the Natural History Museum London (NHM)); two males, “Turkey: | Gumusane, | Soganli Gecidi, 7-7500’. | 25. vii.

Table 1 Locality data for the specimens sequenced and specimens used for bioacoustics evaluation

Voucher ID	Species	Location	Geographical position	GenBank accession nos.		
				COI	ITS1	ITS2
out1	*Saga pedo* (Pallas, 1771)	Bulgaria: E Stara Planina Mts, Zeravna vill, 900 m	42.8427 N 26.4519 E	KT936310	KT823256	KT823233
out2	*Platycleis* (*Squamiana*) sp.	Turkey: Zara-Susehri road, 1650 m	39.5556 N 37.9161 E	KT936311	KT358278	KT358337
out3	*Onconotus servillei* Fisher von Waldheim, 1846	Bulgaria: Kapitan Dimitrovo vill.	43.95 N 27.7 E	KT936312	KT358279	KT358338
out4	*Amphiestris baetica* (Rambur, 1838)	Spain: Cultivo hija de otra de los Barrios, Cadiz	36.32 N 6.17 W	KT936313	KT358280	KT358339
out5	*Paratlanticus ussuriensis* (Uvarov, 1926)	Russia: Primorsky Krai, Lazovskii Natural Reserve, Korpad	43.557 N 133.5726 E	KT936314	KT358336	KT358340
tam1a	*T. armeniaca* complex	Turkey: Horasan-Agri, Saclidag Pass, 2160 m	39.8747 N 42.3856 E	KT358223	KT358281	KT358341
tam1b	*T. armeniaca* complex	Turkey: Horasan-Agri, Saclidag Pass, 2160 m	39.8747 N 42.3856 E	KT358224	KT358282	KT358342
tam2a	*T. armeniaca* complex	Turkey: Horasan-Agri, Savsat-Ardahan road, 1630 m	41.2312 N 42.4338 E	KT358225	KT358283	KT358343
–	*T. armeniaca* complex	Turkey: Pulumur, 1818 m	39.51934 N 39.87208 E	–	–	–
–	*T. armeniaca* complex	Turkey: Ispir, 1900 m	40.583 N 40.883 E	–	–	–
–	*T. armeniaca* complex	Armenia: above Djermuk, 2400 m	39.86365 N 45.69338 E	–	–	–
–	*T. armeniaca* complex	Armenia: E Saravan, 2290 m	39. 68,531 N 45.70808 E	–	–	–
–	*T. armeniaca* complex	Armenia: Shorja near Sevan Lake, 1965 m	40. 50,393 N 45.30347 E	–	–	–
–	*T. armeniaca* complex	Armenia: Lermontovo vill., 1850 m	40.74874 N 44.66132 E	–	–	–
–	*T. armeniaca* complex	Armenia: N of Vardaghbyur, 2015 m	40.99647 N 43.88796 E	–	–	–
tca1	*T. cantans* (Fuessly, 1775)	Hungary: Borzsony Mts	47.55 N 19.00 E	KT358226	KT358284	KT358344
tca2a	*T. cantans* (Fuessly, 1775)	Poland: OPN, Dolina Sąpowska	50.1236 N 19.4845 E	KT358227	KT358285	KT358345
tca2b	*T. cantans* (Fuessly, 1775)	Poland: OPN, Dolina Sąpowska	50.1236 N 19.4845 E	KT358228	KT358286	KT358346
tca2c	*T. cantans* (Fuessly, 1775)	Poland: OPN, Dolina Sąpowska	50.1236 N 19.4845 E	KT358229	KT358287	KT358347
tca2d	*T. cantans* (Fuessly, 1775)	Poland: OPN, Dolina Sąpowska	50.1236 N 19.4845 E	KT358230	KT358288	KT358348
tca2e	*T. cantans* (Fuessly, 1775)	Poland: OPN, Dolina Sąpowska	50.1236 N 19.4845 E	KT358231	KT358289	KT358349
tca2f	*T. cantans* (Fuessly, 1775)	Poland: OPN, Dolina Sąpowska	50.1236 N 19.4845 E	KT358232	KT358290	KT358350
tca3a	*T. cantans* (Fuessly, 1775)	Romania: Lepsa	45.57 N 26.34 E	KT358244	KT358291	KT358351
tca3b	*T. cantans* (Fuessly, 1775)	Romania: Lepsa	45.57 N 26.34 E	KT358245	KT358292	KT358352
tca3c	*T. cantans* (Fuessly, 1775)	Romania: Lepsa	45.57 N 26.34 E	KT358247	KT358293	KT358353
tca3d	*T. cantans* (Fuessly, 1775)	Romania: Lepsa	45.57 N 26.34 E	KT358246	KT358294	KT358357
tct3	*T. cantans* (Fuessly, 1775)	China: Xinjiang, near Tianchi (or Tienchi/Heaven Lake) in Tianshan Mts. near mountain of Bogda Feng, 2000 m	43.9 N 88.117 E	KT358235	KT358297	KT358357
tct1	*T. cantans* (Fuessly, 1775)	Kyrgyzstan: Isik Ata	42.53 N 74.51 E	KT358233	KT358295	KT358355
–	*T. caudata* (Charpentier, 1842)	Turkey: Ispir, 1900 m	40.583 N 40.883 E	–	–	–

Table 1 (continued)

Voucher ID	Species	Location	Geographical position	GenBank accession nos.		
				COI	ITS1	ITS2
–	*T. caudata* (Charpentier, 1842)	Armenia: Gorhajk near Vorotan Dam, 2120 m	39.68521 N 45.78486 E	–	–	–
tct2	*T. caudata* (Charpentier, 1842)	Bulgaria: Byala	43.4717 N 25.7696 E	KT358234	KT358296	KT358356
tdm	*T. uvarovi* Ebner, 1946	Russia: Primorsky Krai, Ussuri River, Gornye Kluchi (Shamkovka)	45.20 N 134.40 E	KT358236	KT358298	KT358358
tmo1a	*T.* cf. *longealata*	Morocco: S Ajabo, 1360 m	33.0659 N 5.4086 W	KT358254	KT358299	KT358359
tmo1b	*T.* cf. *longealata*	Morocco: S Ajabo, 1360 m	33.0659 N 5.4086 W	KT358252	KT358300	KT358360
tmo1c	*T.* cf. *longealata*	Morocco: S Ajabo, 1360 m	33.0659 N 5.4086 W	KT358253	KT358301	KT358361
tmo2a	*T.* cf. *longealata*	Morocco: NW Khenifra, 1100 m	33.1377 N 5.9235 W	KT358261	KT358302	KT358362
tmo2b	*T.* cf. *longealata*	Morocco: NW Khenifra, 1100 m	33.1377 N 5.9235 W	KT358262	KT358303	KT358363
tmo2c	*T.* cf. *vaucheriana*	Morocco: NW Khenifra, 1100 m	33.1377 N 5.9235 W	KT358248	KT358304	KT358364
tmo3a	*T.* cf. *vaucheriana*	Morocco: near El Kebab, 966 m	32.7569 N 5.6451 W	KT358257	KT358305	KT358365
tmo3b	*T.* cf. *vaucheriana*	Morocco: near El Kebab, 966 m	32.7569 N 5.6451 W	KT358251	KT358306	KT358366
tmo3c	*T.* cf. *vaucheriana*	Morocco: near El Kebab, 966 m	32.7569 N 5.6451 W	KT358249	KT358307	KT358367
tmo3d	*T.* cf. *vaucheriana*	Morocco: near El Kebab, 966 m	32.7569 N 5.6451 W	KT358250	KT358308	KT358368
tmo3e	*T.* cf. *vaucheriana*	Morocco: near El Kebab, 966 m	32.7569 N 5.6451 W	KT358242	KT358309	KT358369
tmo4a	*T.* cf. *vaucheriana*	Morocco: SE Thar Es-Souk, 650 m	34.6585 N 4.2417 W	KT358258	KT358310	KT358370
tmo4b	*T.* cf. *vaucheriana*	Morocco: SE Thar Es-Souk, 650 m	34.6585 N 4.2417 W	KT358260	KT358311	KT358371
tmo5a	*T.* cf. *viridissima*	Morocco: S Aïn Zora, 835 m	34.5708 N 3.6657 W	KT358256	KT358312	KT358372
tmo5b	*T.* cf. *viridissima*	Morocco: S Aïn Zora, 835 m	34.5708 N 3.6657 W	KT358263	KT358313	KT358373
tmo5c	*T.* cf. *viridissima*	Morocco: S Aïn Zora, 835 m	34.5708 N 3.6657 W	KT358255	KT358314	KT358374
tmo5d	*T.* cf. *viridissima*	Morocco: S Aïn Zora, 835 m	34.5708 N 3.6657 W	KT358265	KT358315	KT358375
tmo5e	*T.* cf. *viridissima*	Morocco: S Aïn Zora, 835 m	34.5708 N 3.6657 W	KT358264	KT358316	KT358376
tmo6a	*T.* cf. *vaucheriana*	Morocco: Bouchfaa W Taza, 675 m	34.0830 N 4.2996 W	KT358239	KT358317	KT358377
tmo6b	*T.* cf. *vaucheriana*	Morocco: Bouchfaa W Taza, 675 m	34.0830 N 4.2996 W	KT358238	KT358318	KT358378
tmo7a	*T.* sp. aff. *viridissima*	Morocco: E Azrou, 1520 m	33.4259 N 5.1926 W	KT358268	KT358319	KT358379
tmo7b	*T.* sp. aff. *viridissima*	Morocco: E Azrou, 1520 m	33.4259 N 5.1926 W	KT358266	KT358320	KT358380
tmo7c	*T.* sp. aff. *viridissima*	Morocco: E Azrou, 1520 m	33.4259 N 5.1926 W	KT358267	KT358321	KT358381
tmo7d	*T.* sp. aff. *viridissima*	Morocco: E Azrou, 1520 m	33.4259 N 5.1926 W	KT358243	KT358322	KT358382
tmo8a	*T.* cf. *vaucheriana*	Morocco: Tilougguite Pass, 1570 m	32.0852 N 6.3003 W	KT358241	KT358323	KT358383
tmo8b	*T.* cf. *vaucheriana*	Morocco: Tilougguite Pass, 1570 m	32.0852 N 6.3003 W	KT358240	KT358324	KT358384
tmo9	*T.* cf. *vaucheriana*	Morocco: SW Derdara, 400 m	35.0896 N 5.3074 W	KT358259	KT358325	KT358385

Table 1 (continued)

Voucher ID	Species	Location	Geographical position	GenBank accession nos.		
				COI	ITS1	ITS2
–	*T.* cf. *vaucheriana*	Morocco: N Fes, 20 m	34.47138 N 5.38195 W	–	–	–
–	*T.* cf. *vaucheriana*	Morocco: Tilougguite Pass, 1570 m	32.08519 N 6.30028 W	–	–	–
tvi1	*T. viridissima* (Linnaeus, 1758)	Kyrgyzstan: Ata Arche	42.3842 N 74.2848 E	KT358273	KT358326	KT358386
tvi2	*T. viridissima* (Linnaeus, 1758)	Spain: Isik Ata, Montes de Toledo	39.3045 N 04.4353 W	KT358237	KT358327	KT358387
tvi3a	*T. viridissima* (Linnaeus, 1758)	Ukraine: Donetsk Region	48.14 N 37.74 E	KT358274	KT358328	KT358388
tvi3b	*T. viridissima* (Linnaeus, 1758)	Ukraine: Donetsk Region	48.14 N 37.74 E	KT358275	KT358329	KT358389
tvi3c	*T. viridissima* (Linnaeus, 1758)	Ukraine: Donetsk Region	48.14 N 37.74 E	KT358276	KT358330	KT358390
tvi3d	*T. viridissima* (Linnaeus, 1758)	Ukraine: Donetsk Region	48.14 N 37.74 E	KT358277	KT358331	KT358391
tvi4	*T. viridissima* (Linnaeus, 1758)	Turkey: Yanikcay, 1920 m	38.2547 N 42.8978 E	KT358269	KT358332	KT358392
tvi5a	*T. viridissima* (Linnaeus, 1758)	Bulgaria: Varna, Botanical Garden	43.2374 N 28.003 E	KT358272	KT358333	KT358393
tvi5b	*T. viridissima* (Linnaeus, 1758)	Bulgaria: Haskovo, Perperikon Ruins	41.715 N 25.4657 E	KT358270	KT358334	KT358394
tvi5c	*T. viridissima* (Linnaeus, 1758)	Bulgaria: Dobrich, Bolata Bay	43.3838 N 28.4715 E	KT358271	KT358335	KT358395

All sequences are submitted to the NCBI GenBank

1960. | K. M. Guichard | & D. H. Harvey. | B.M. 1960-364" (NHM).

Tettigonia armeniaca stat. nov.—two females (not identified), "Isbisu (Gov. Eriwan)" (NHMW); female (not identified), "Bakurian" [Georgia] (NHMW); male (not identified), "Kasikoparan" [Turkey] (NHMW); male (not identified), "Soganli Gecidi" [Turkey] (NHMW)

T. cantans (Fuessly, 1775)—male, "Karnten 1927 | Vellacher Toschna, 3. ix. | coll. R. Ebner" (NHMW)

T. caudata (Charpentier, 1842)—male, "Jasenova, Jugoslawien" (NHMW); male, "Pirot" (NHMW); male, "Walouiki, R. m. | Velitchkovsky" [Ukraine] (NHMW); male, "Gegend v. Wien | Von Hn. Turk | Coll. Br. v. W." (NHMW); male, "Eriwan-Tiflis" (NHMW); male, "Poin-Shaval, Elbrus | Funke leg." (NHMW); male, "Persia s.- | Elburs | Rehne-Demavend | ca. 2700–3600 m | 20–27. vii. 1936" (NHMW); male, "Sabzawaran | 12. v. 50 / Ö sterr. | Iran exped. 1950" (NHMW); male, "Afghanistan | Chira | Hr. v. Pleson | Coll. Br. v. W." (NHMW)

Tettigonia lozanoi (Bolívar, 1914)—male, Aguelman (NHM)

Tettigonia uvarovi Ebner, 1946—male, holotype, Siberia (NHMW)

Tettigonia vaucheriana Pictet, 1888—male, "Morocco, Azrou, 1200–1400 m, 28. v.-1. vi. 1930. Ebner" (NHMW); female, "Atlas, Asni | 1200 m, 23–30. vi.' 30. | Ebner" (NHMW)

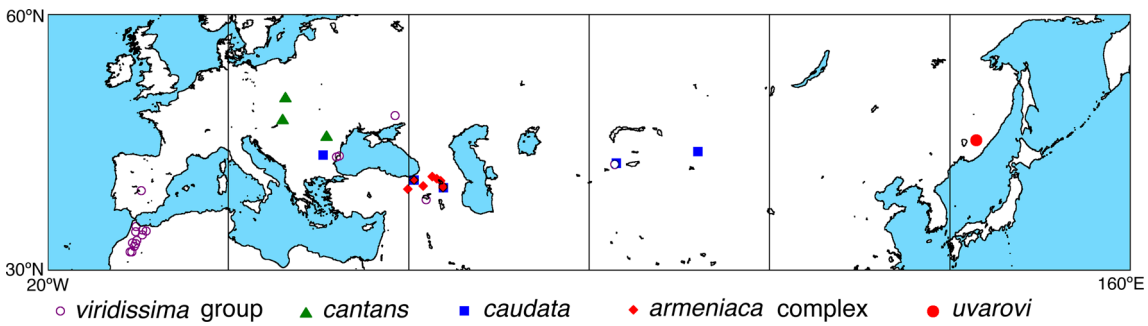

Fig. 1 Map showing the sampling sites for *Tettigonia*

According to the state of knowledge, taxonomic recognition and morphological similarities, we divided *Tettigonia* taxa into three groups:

1. Commonly recognized taxa: well-studied *T. viridissima* (Linnaeus, 1758), *T. caudata* (Charpentier, 1842) s. str., and *T. cantans* (Fuessly, 1775) of the Western Palaearctic.
2. Taxa that have been recently described and only partially studied: *Tettigonia dolichoptera maritima* Storozhenko, 1994 = *T. uvarovi* Ebner, 1946 (see Storozhenko et al. 2015). Described from the Russian Far East, this subspecies was thought to differ in the length of the pronotum and tegmina from the nominotypical form from South Korea. However, many South Korean specimens are similar to *T. uvarovi* in their dimensions (Rhee 2013), and only the most long-winged ones are now assumed to represent *T. dolichoptera* (Storozhenko et al. 2015). In any case, this representative of the Eastern Palaearctic fauna may provide clues as to the relationships and phylogeographic connections of the east and west Palaearctic lineages of *Tettigonia*.
3. Poorly known sibling species termed here as follows:

   (a) The *T. armeniaca* complex. Upon sampling of a specific shorter-winged *Tettigonia*, resembling *T. caudata* in terms of many morphological features, which occurs from Eastern Anatolia to Southern Caucasus and possibly further to Kyrgyzstan (own unpublished information), we failed to definitely outline morpho-units that fit each of the taxa *T. caudata armeniaca* Tarbinsky, 1940 (presently a synonym of *T. caudata* s. str.), *T. acutipennis* Ebner, 1946, and *Tettigonia turcica* Ramme, 1951. This complex has been previously defined by weak (but present) black coloration at the base of ventral post-femoral spines and more or less shortened wings.

   (b) The *T. vaucheriana* complex. A multitude of forms has been described from northwestern Africa, differing mostly in size, length of the forewings, and relative width of the scapus (front border of the vertex bordering the frons) (*T. vaucheriana* Pictet, 1888 = *Tettigonia maroccana* Bolívar, 1893, syn.; *T. lozanoi* (Bolívar, 1914); *Tettigonia longealata* Chopard, 1937; *Tettigonia krugeri* Massa, 1998). Some of them resemble *T. viridissima*, which has been recorded from North Africa. Upon extensive sampling in Morocco and comparison of museum specimens, we observed a significant overlap between populations, with extreme examples ranging from a slender body shape with long wings (*T. viridissima* type) to a stout body with long wings (*T. longealata*) or short wings (*T. vaucheriana*, *T. lozanoi*) as this has already been noted by Pinedo (1985).

Genomic sampling

DNA extraction was performed using NucleoSpin® Tissue Kits (*Macherey-Nagel, Düren*, Germany) according to the standard protocol. DNA was used as a PCR template to amplify four genetic markers, including mitochondrial and nuclear genes. These were (1) partial cytochrome c oxidase subunit I (COI), (2) partial sequences of the first internal transcribed spacer (ITS1) of the nuclear ribosomal gene cluster, and (3) partial sequences of the second internal transcribed spacer (ITS2) of the nuclear ribosomal gene cluster. The COI gene was amplified with the primers LCO [5′ GGT CAA CAA ATC ATA AAG ATA TTG G 3′] and HCO [5′ TAA ACT TCA GGG TGA CCA AAA AAT CA 3′] (Folmer et al. 1994). For nuclear DNA, ITS1 regions were PCR amplified using the primers 18S-28S [5′ TAG AGG AAG TAA AAG TCG 3′] (Weekers et al. 2001) and ITS-R1 [5′ CAT TGA CCC ACG AGC C 3′] (Ullrich et al. 2010), whereas ITS2 regions were amplified using the primers ITS2-28S [5′ GGA TCG ATG AAG AAC G 3′] and 28S-18S [5′ GCT TAA ATT CAG CGG 3′] (Weekers et al. 2001).

PCR was performed in 30-μL reaction volumes, which comprised 10 pmol of each primer, 10 mM of each dNTP, 25 mM $MgCl_2$, 2.5 μL 10× PCR buffer, 1 U *Taq* polymerase (EURx, Gdańsk, Poland), and sterile H_2O.

To amplify COI, we used the following PCR protocol: 35 cycles at 95 °C for 50 s, 50 °C for 1 min and 72 °C for 1 min, with the final extension at 72 °C for 6 min. PCR amplification of ITS1 and ITS2 consisted of 25 cycles at 95 °C for 1 min, 52 °C for 1 min 50 s, and 72 °C for 2 min, with the final extension at 72 °C for 10 min. PCR products were purified with the Gene MATRIX PCR/DNA Clean-Up Purification Kit (EURx, Poland, following the standard protocol) and sequenced directly. Purified DNA was sequenced in both directions using the same primers as for PCR and the Big Dye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions.

Phylogenetic analyses

DNA sequences were edited and compiled using Muscle (Edgar 2004). To test for pseudogenes, coding sequences (COI) were translated into protein with MEGA 6 (Tamura et al. 2013) using the standard invertebrate mitochondrial genetic code. No stop codons were observed. Nucleotide composition homogeneity within genes was tested with PAUP* 4.0b10 (Swofford 2002). Mean net genetic distances among clades were calculated using MEGA 6 (Tamura et al. 2013)

within the Kimura two-parameter model (K2P, standard errors (SE) were obtained by bootstrapping with 1000 replicates).

Two different phylogenetic methods, maximum likelihood (ML) and Bayesian inference (BI), were used to infer evolutionary relationships. Following independent analysis for each COI, ITS1, and ITS2 dataset, the COI and ITS1 + ITS2 datasets were concatenated and further analyses were performed using the combined matrix. Evolutionary models for each dataset and combined dataset were selected using MrModeltest 2.3 (Nylander 2004) with the Akaike information criterion (Akaike 1974). Support for nodes in ML analysis was assessed with non-parametric bootstrapping (BP) using Phyml (Guindon and Gascuel 2003) with 1000 pseduoreplicates and ten random BioNJ trees, and parameters were estimated from each dataset within the model selected for the original dataset. BI of phylogenetic relationships using Metropolis-coupled Monte Carlo Markov chain (mcmc) simulation was performed with MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Huelsenbeck et al. 2001). Posterior probabilities were based on two independent MCM runs, each composed of four chains (three heated chains and one cold chain). The mcmc simulations were run for 10,000,000 generations with sampling every 100 generations. The convergence of analyses was validated by monitoring likelihood values graphically using Tracer (Rambaut and Drummond 2007), and trees prior to stationarity were discarded as burn-in. A 50% majority-rule consensus tree was constructed from the remaining trees to estimate posterior probabilities (PPs). Phylogenetic trees were produced using TreeView (Page 1996) and FigTree software (Rambaut 2008).

Bioacoustic evaluations

Male songs were recorded under different environmental conditions using the following equipment: (1) Knowles BT-1759-000 electret condenser microphone with a sensitivity of −60 ± 3 dB re 1 V/μbar at 1 kHz and with a frequency response roll-off of about 10 kHz and cutoff at over 45 kHz (data combined from Irie 1995 and W. Schulze, Friedrich-Alexander-Universität Erlangen-Nürnberg, personal communication), equipped with a custom-made preamplifier connected to a PC through an external soundcard (Transit USB, "M-Audio") (48/96-kHz sampling rate), used in the lab; (2) Pettersson D500 external microphone with a frequency range corresponding to that of the Pettersson D500x recorder, being between 1 (−6 dB)–2 kHz (−3 dB) and 190 kHz (500-kHz sampling rate) (Lars Pettersson, personal communication), connected to a ZOOM H2 or ZOOM H4 handy recorder (Zoom Corporation) (96-kHz sampling rate), used in captivity and in nature; (3) UHER M645 microphone with a frequency response flat up to 20 kHz connected to a UHER 4200 IC tape recorder; and (4) Brüel and Kjaer 4135 microphone connected to a RACAL store 4DS tape recorder.

The *bioacoustic terminology* used in this study is as follows (based on Ragge and Reynolds 1998, modified from Chobanov et al. 2014): calling song—the song produced by an isolated male; echeme—a first-order grouping of syllables; echeme duration—the time measured from the beginning of the first to the end of the last syllable; echeme period—the span including an echeme and the following interval; syllable—the sound produced by one opening-and-closing movement of the tegmina; syllable period—the span including a syllable and the following interval, usually measured between syllable peaks; syllable repetition rate—reciprocal of the syllable period (unit Hz = 1/s); diplosyllables = disyllabic echemes—two syllables separated from the neighboring diplosyllables by longer silent intervals than those within each diplosyllable; duty cycle—during singing activity, the proportion of time spent actually singing: echeme duration divided by the echeme period (in *T. viridissima* duration of echeme sequences divided by duration of acoustic activity); and chirp—an isolated acoustic event regardless of its structure. Temperature during the recordings varied, but for evaluation, we used only temperature-independent structures (duty cycle) and relationships (relationship between temperature-dependent chirp duration and temperature-dependent interval duration). Differences between a daytime song consisting of chirps and a continuous nighttime song as in *T. cantans* were not observed in *T. armeniaca* complex nor in any other *Tettigonia* species.

We concentrate to the poorly studied groups of the here named *T. armeniaca* complex and *T. vaucheriana* complex. Altogether, nine continuous recordings under different conditions (temperature, time of the day, different male individual) from six remote localities of males, representing different morphotypes of the *T. vaucheriana* complex, were studied (see Fig. 2). From the *T. armeniaca* complex, we studied, respectively, 34 recordings from ten localities (partly represented in Fig. 3). Own data for the rest of the studied taxa were supplemented with published recordings and song measurements. Recordings of *T. cantans* and *T. caudata caudata* from Massa et al. (2012) are used for comparative purposes in the figures (see "Results" section).

The recordings used for duty cycle measurements included those made by the present authors as well as recordings from several published sound sources (Grein 1984; Bonnet 1995; Kleukers et al. 1997; Ragge and Reynolds 1998; Nielsen 2000; Odé and Fontana 2002; Bellmann 2004; Barataud 2007; Roesti and Keist 2009; Massa et al. 2012; Kocarek et al. 2013; Gomboc and Segula 2014) and from the Internet (SYSTAX 2015; data provided by G. Schmidt).

Processing of sound files, measurements, and preparation of oscillograms were performed with Audacity 2.0.3 (Audacity team 1999–2013), BatSound 4.1.4 (Pettersson Electronics and Acoustics AB 1996–2010), and Amadeus II (Martin Hairer; http://www.hairersoft.com).

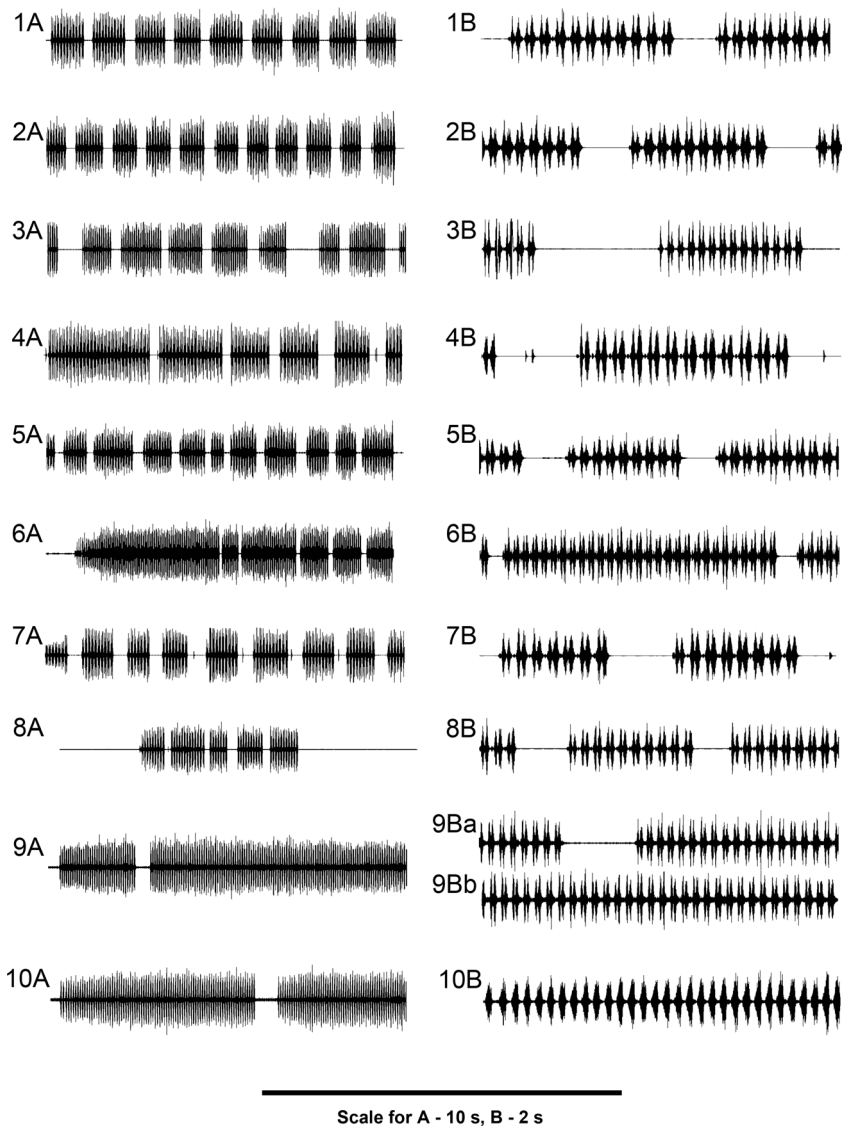

Fig. 2 Oscillograms of the song of the *Tettigonia viridissima* group (*1–9*) and *T. cantans* (*10*) recorded at two speeds: *1 T.* cf. *longealata* (MO: Ajabo, $T = 20$ °C), *2 T.* cf. *vaucheriana* (MO: N Fes, $T = 20$ °C), *3 T.* cf. *vaucheriana* (MO: Bouchfaa W of Taza, $T = 21$ °C), *4 T.* cf. *vaucheriana* (MO: Tilougguite Pass, $T = 23$ °C), *5 T.* cf. *vaucheriana* and cf. *longealata* (MO: El Kebab, $T = 25$ °C), *6 T.* cf. *vaucheriana* (MO: El Kebab, $T = 28–30$ °C), *7 T.* cf. *viridissima* (MO: S Aïn Zora, $T = 22$ °C), *8 T.* cf. *viridissima* (MO: S Aïn Zora, $T = 25$ °C), *9 T. viridissima* (BG: Sofia, $T = 27$ °C), and *10 T. cantans* (IT: Val Malene; from Massa et al. 2012, $T = 15$ °C)). *Scale bar* for *A* is 10 s and for *B* 2 s

Results

Phylogenetic reconstruction

COI, ITS1, and ITS2 genes are standard markers used in phylogenetic studies of insects, which in many cases have proved informative on a specific and generic level. We obtained the following fragments: 547 bp for COI, 400 bp for ITS1, and 420 bp for ITS2 (including gaps and variable regions). No indels were observed in the COI fragment.

The congruency of COI, ITS1, and ITS2 ($p = 0.99$) allowed for these markers to be combined into a single matrix. The resulting 1367 bp matrix was obtained after alignment and trimming, containing 20% variable and 13% parsimony informative sites. MrModeltest identified the GTR + G model (gamma distribution shape parameter $G = 0.94$; $-\ln L = 12{,}691.23$; AIC = 25,400.47) as the best nucleotide substitution model for ML and BI analyses.

Phylogenies reconstructed based on the combined data using ML and Bayesian methods (Fig. 4) showed similar topologies. The tree inferred from COI + ITS1 + ITS2 sequences (Fig. 4) showed that *Tettigonia* taxa are grouped into three main clades. Clade "A" includes all sampled populations of *T. viridissima* and all northwestern African specimens of the taxon referred to in this paper as the *T. vaucheriana* complex. Clade "B" is composed of *T. caudata*, *T. uvarovi*, and what is here referred to as the *T. armeniaca* complex. Clade "C" is composed of all the *T. cantans* samples in our dataset.

The genetic distances between and within clades for all genes are presented in Tables 2 and 3, respectively. Genetic distances between major clades were similar for COI (1–4%) and for ITS (1–3%). The genetic distances between ingroup species were very low for COI (0–2%).

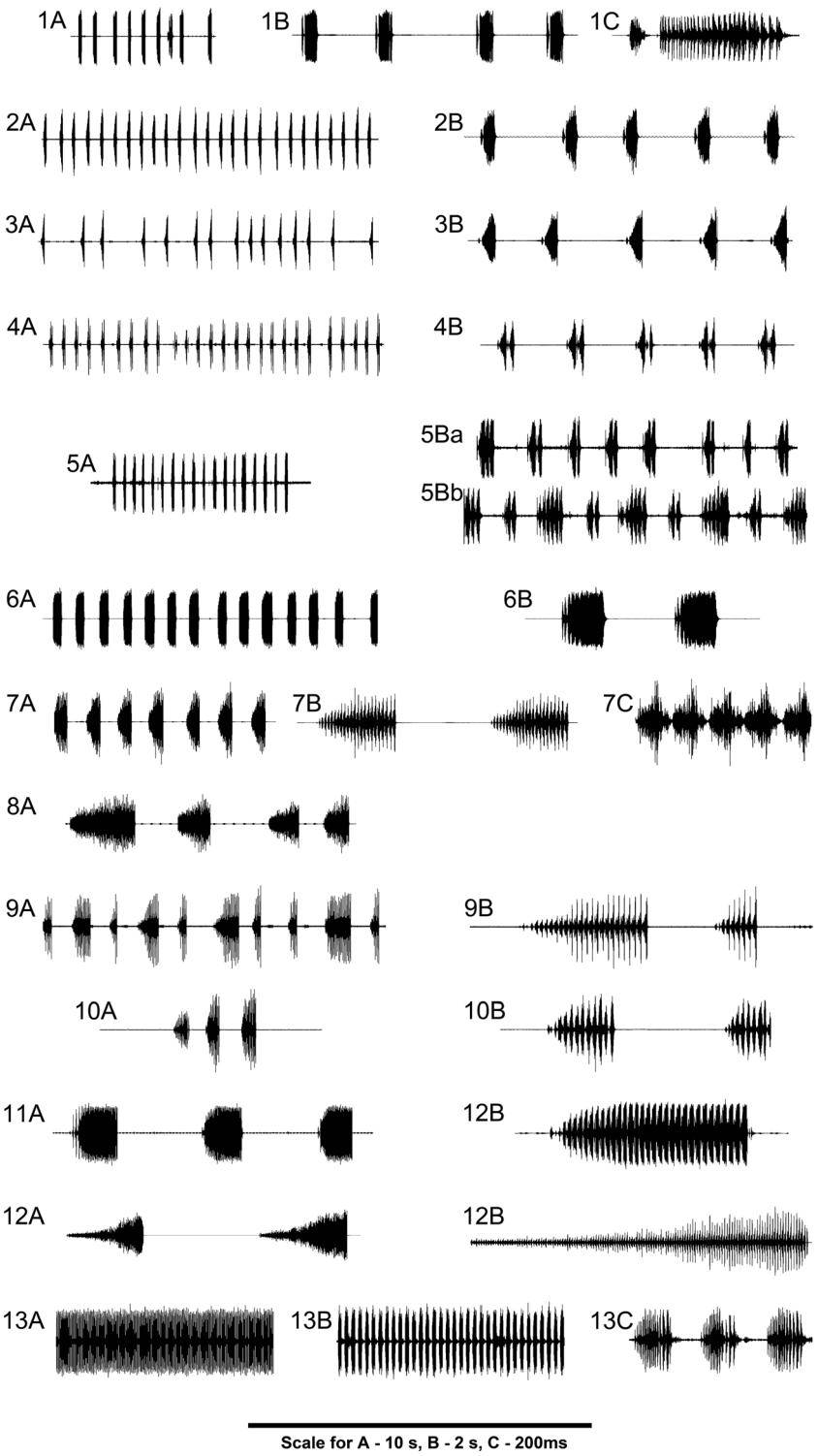

Fig. 3 Oscillograms of songs of the *Tettigonia armeniaca* complex (*1–11*), *T. caudata* (*12*), and *T. uvarovi* (*13*) recorded at two or three speeds: *1 T. armeniaca* (AM: Djermuk, $T = 19$ °C), *2 T. armeniaca* (TR: Saclidag Pass, $T = 21.5$ °C), *3 T. armeniaca* (TR: Ispir, $T = 17$ °C) (monosyllabic type), *4 T. armeniaca* (TR: Ispir, $T = 17$ °C) (disyllabic type), *5 T. armeniaca* (AM: Saravan, $T = 20–25$ °C) (outdoor recording), *6 T. armeniaca* (AM: Lermontovo vill., $T = 20$ °C), *7 T. armeniaca* (TR: Savsat–Ardahan, $T = 26$ °C), *8 T. armeniaca* (TR: Savsat–Ardahan, $T = 26$ °C) (variable echeme length), *9 T. armeniaca* (TR: Ispir, $T = 17$ °C) (polysyllabic type of variable length), *10 T. armeniaca* (TR: Pulumur, $T = 20–22$ °C) (shorter echemes), *11 T. armeniaca* (TR: Pulumur, $T = 20–22$ °C) (the same specimen as in *10* longer echemes), *12 T. caudata* (GR: Drama; from Massa et al. 2012, $T = 25$ °C), and *13 T. uvarovi* (South Korea; from Kim 2009 as *T. dolichoptera*; see Rhee 2013). *Scale bar* for *A* is 10 s; for *B*, 2 s; and for C, 200 ms

Bioacoustic evaluation and morphological characters

The three bioacoustically well-distinguished species (see "Introduction" section) belong to three well-outlined clusters in our study. In addition to the previously known acoustic diversity in Central Europe, we found striking examples of variation among populations with uniform morphology, as well as uniform song patterns among morphologically distinct populations hitherto classified as different species.

Clade A (Figs. 4 and 5j–m) comprises haplotypes with low (COI) to very low (ITS) ingroup genetic distances. This clade includes populations from a large portion of the range of

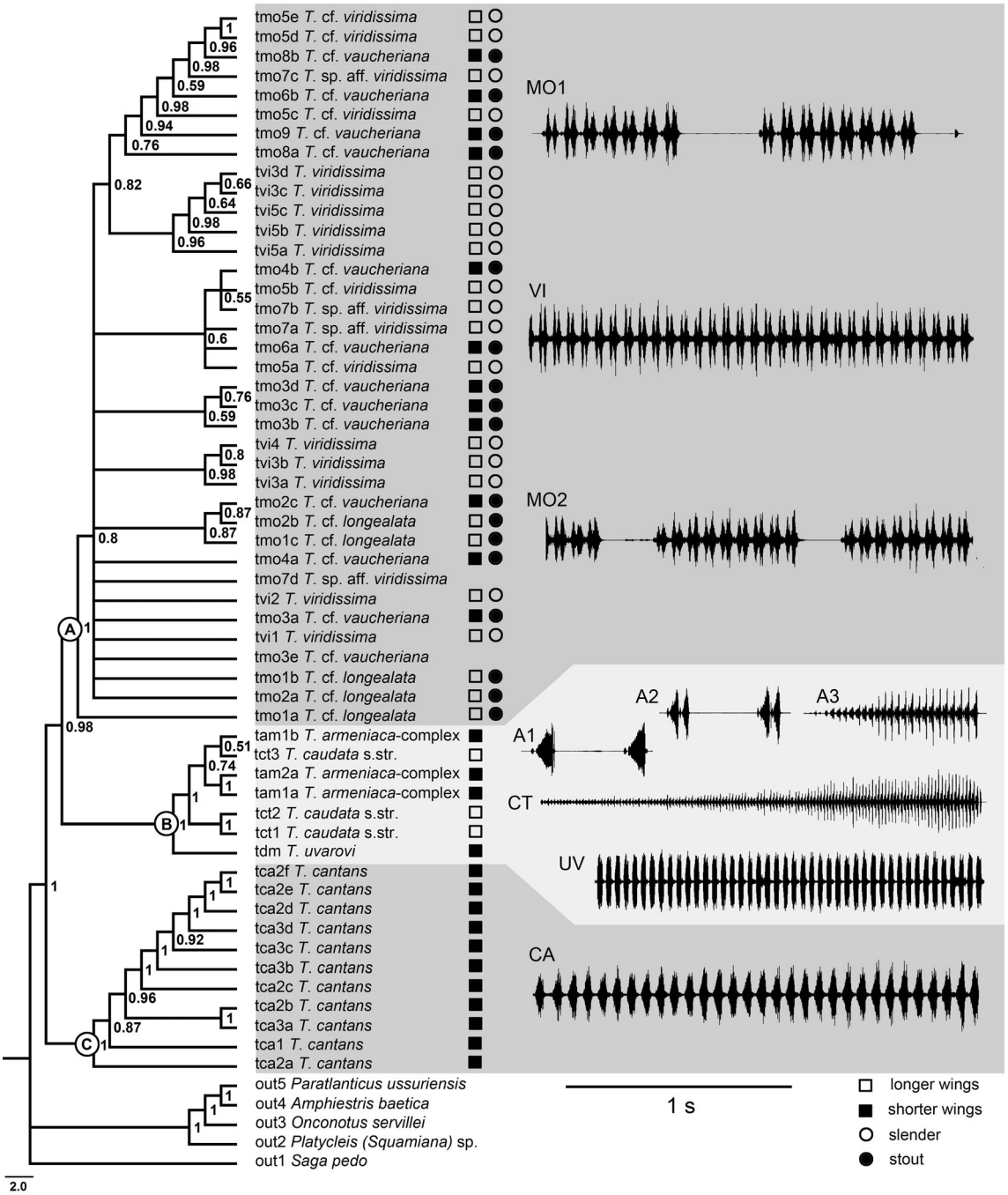

Fig. 4 Phylogenetic tree of the genus *Tettigonia* based on BI analysis of concatenated COI-ITS1-ITS2 sequences. BI posterior probability (PP) values are shown near resolved branches (only support values above 0.50). Species groups, as defined by genetic and morpho-acoustic data, are distinctly *shaded*, and the respective branches are marked with an *open circle* and a *capital letter* as follows: "*A*"—*T. viridissima* group, "*B*"—*T. caudata* group, and "*C*"—*T. cantans* group. Haplotype codes correspond to Table 1 in the Supplement, followed by morphological identification. *Squares on the right side of names* correspond to relative wing length: *filled squares* short wings and *open squares* long wings; *circles* for the *T. viridissima* group correspond to relative body shape: *filled circles* larger and stouter body and *open circles* smaller and more slender body. A partial oscillogram with the main song types is presented for each group: *MO* Moroccan populations of the *T. viridissima* group; *VI T. viridissima* (specimen from Bulgaria); *A1*, *A2*, and *A3* three song types of different specimens of the *T. armeniaca* complex from the Erzurum region (Ispir); *CT T. caudata* (Greece, Drama; from Massa et al. 2012); *DM T. uvarovi* (S Korea; from Kim et al. 2009); *CA T. cantans* (Italy, Val Malene; from Massa et al. 2012). *Scale bar* corresponds to 1 s of recording (for temperature during recordings, see Figs. 2 and 3)

T. viridissima as well as all populations sampled in Northwestern Africa that may be identified as one of *T. vaucheriana*, *T. lozanoi*, *T. longealata*, *T. krugeri*, and *T. viridissima*. All these taxa were described in terms of

Table 2 Net mean genetic distances (%) between *Tettigonia* clades for mitochondrial (COI) and nuclear (ITS1 + ITS2) genes

		T1	T2	T3	T4
COI clades					
T. viridissima + *T vaucheriana* complex	T1				
T. uvarovi	T2	0.02			
T. armeniaca complex + *T. caudata* s. str.	T3	0.03	0.02		
T. caudata s. str.	T4	0.04	0.03	0.02	
T. cantans	T5	0.01	0.03	0.03	0.04
ITS1 + ITS2 clades					
T. viridissima + *T vaucheriana* complex	T1	-	-		
T. uvarovi	T2	0.01	-		
T. armeniaca complex + *T. caudata* s. str.	T3	0.01	0.02		
T. caudata s. str.	T4	0.02	0.02	0.01	
T. cantans	T5	0.02	0.03	0.01	0.01

differences in body size, relative length of the tegmina (see open and closed symbols designating each specimen in Fig. 4), and some additional features, such as the width of the fastigium of the vertex (according to our observations in this group, a wide fastigium corresponds to large, stout body and vice versa). Despite these "strict" differences, we failed to clearly outline taxa as wide variation was observed between populations, and animals with both long and short wings occurred together in some areas. All sampled populations and studied museum specimens were compared (also with descriptions), and we did not find differences in the shape of male cerci and genitalia (titillators), female subgenital plate, or other species-specific characters.

All studied individuals from the sampled populations of clade A showed the same song pattern—sequences of disyllabic echemes of variable length separated by short intervals (see Fig. 2). Large intraindividual variation in the echeme sequence length was also observed. The fine structure of the song fully corresponded to that of *T. viridissima* though the latter species usually produced longer echeme sequences. Yet, a large overlap was detected between the song duty cycles (calculated using echemes and echeme intervals) of the northwestern African populations and *T. viridissima* (Fig. 6). The genetic data, showing low genetic diversification, support the phenotypic similarities.

Clade B (Fig. 4) is formed by *T. uvarovi* (Fig. 5c, d), *T. caudata* (Fig. 5e), and the *T. armeniaca* complex (Fig. 5f–i). *T. uvarovi* is a well-characterized species, morphologically resembling *T. viridissima*, with a song more similar to that of *T. cantans* (Rhee 2013). *T. caudata* is also a well-studied species, though this only applies to its nominotypical form, while its relationships with subspecific taxa are vague. Its typical song, consisting of long echemes (1–10 s), was recorded from Switzerland in the west (Roesti and Keist 2009), through Europe and Anatolia, to China (Xinjiang) in the east (Fan et al. 2013). The *T. armeniaca* complex as here regarded concerns populations sampled in the Transcaucasus and Eastern Anatolia (see Table 1) with specimens fitting either *T. caudata armeniaca*, *T. acutipennis*, or *T. turcica*. All of the latter individuals were characterized in comparison with *T. caudata* s. str. by more or less shortened wings, as well as shorter hind femora, smaller body size, and weaker development of black dots at the base of the ventral spines of the hind femora. Interestingly, although we could not discriminate between populations morphologically, specimens from different localities exhibited a wide variety of song types. The latter caused confusion not only for the fact that specimens with different songs looked the same, but due to the lack of a geographic structuring of the songs. Song types varied from long sequences of isolated syllables to sequences of disyllabic or polysyllabic echemes. However, all songs exhibited approximately the same relationship between chirp duration and chirp interval. Compared with the songs of *T. caudata*, the latter has higher absolute values in both aspects, still preserving the ratio between chirp duration and interval (Fig. 7). Part of the variation is certainly due to different temperatures during recording, but the groups did not overlap despite similar temperature ranges (*T. armeniaca*: 13–27.5 °C; *T. caudata*: 14–27 °C). The duty cycle of the *armeniaca* song (Fig. 6) varied significantly and partly overlapped with that of *T. caudata*.

After observation of dense populations of the *T. armeniaca* complex, we found that the songs of different individuals within the same population may vary to almost the same extent as in general (see Figs. 3 and 7). Rarely, single individuals may also produce a different song by alternating monosyllabic, disyllabic, or polysyllabic echemes within one performance (see Fig. 3 (5Ba, Bb, 9A, 9B)). The genetic structure also showed very low

Table 3 Net mean genetic distances (%) within *Tettigonia* clades for mitochondrial (COI) and nuclear (ITS1 + ITS2) genes

	COI clades	ITS1 + ITS2 clades
T. viridissima + *T vaucheriana* complex	0.02	0.01
T. uvarovi	–	–
T. armeniaca complex + *T. caudata* s. str.	0.01	0.05
T. caudata s. str.	0	0.01
T. cantans	0	0.07

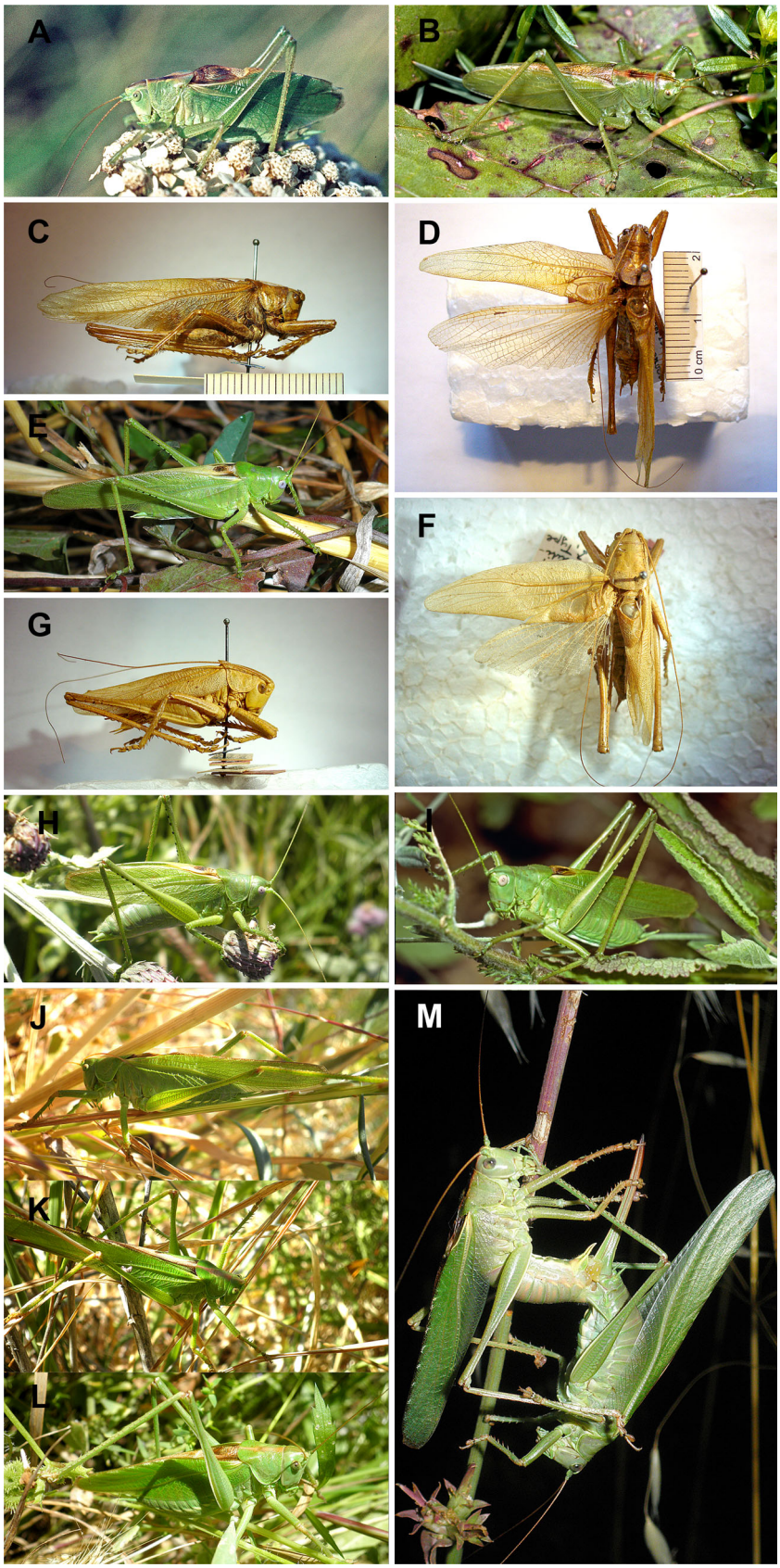

Fig. 5 Appearance of some taxa of Western Palaearctic *Tettigonia* (relative size proportions between photos not retained). **a** *T. cantans*, male, Germany, Gunzenhausen; **b** *T. cantans*, female, Germany, Gunzenhausen; **c** *T. uvarovi* Ebner, 1946—male, holotype, Siberia (NHMW), lateral view; **d** same, dorsal view; **e** *T. caudata*, male, Bulgaria, Russe district, Byala; **f** *T. acutipennis* Ebner, 1946—male, holotype, "Kleinasien 1914 | Marasch, Tölg. | coll. R. Ebner" (NHMW), dorsal view; **g** same, lateral view; **h** *T. armeniaca*, male, Armenia, Djermuk; **i** *T. armeniaca*, male, Turkey, Ispir; **j** *T. viridissima* morphotype of *longealata*, male, Morocco, El Kebab; **k** *T. viridissima* morphotype of *longealata*, female, Morocco, El Kebab; **l** *T. viridissima* morphotype of *vaucheriana*, male, Morocco, El Kebab; and **m** *T. viridissima*, male and female in copula, Bulgaria, Haskovo district, Kostilkovo village

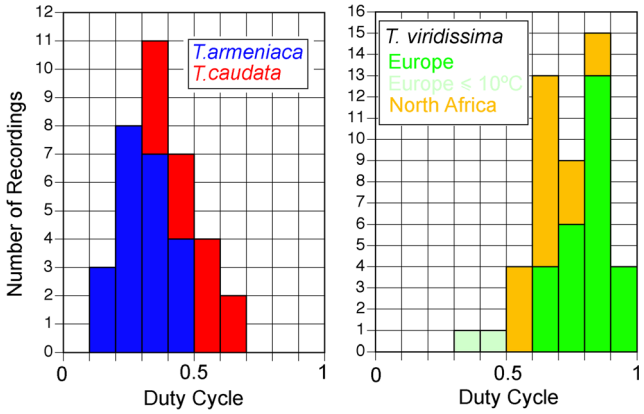

Fig. 6 Comparison of the duty cycle in the songs of the *T. armeniaca* complex and *T. caudata* (*left panel*) and the *Tettigonia viridissima* group (*right panel*)

(almost zero) differentiation for the COI fragment within the *T. armeniaca* complex, while distances within the clade consisting of the *T. armeniaca* complex and *T. caudata* were from very low (COI) to moderate (ITS) (Table 3).

Clade C (Fig. 4) is here represented by a single taxon, *T. cantans* (Fig. 5a, b). Its basal position in the tree supports the suggestion that its song corresponds to an ancestral state for *Tettigonia* due to its simple structure and low female preference towards temporal song parameters (Schul 1998).

Discussion

The genus *Tettigonia* is one of the ecologically most successful Palaearctic groups of bush-crickets, distributed throughout the Palaearctic ecozone. The Green Bush-crickets occur in a wide variety of habitats—from the semideserts of North Africa and Central Asia to Eurasian taiga and the lush meadows of the treeless mountain zone. The low mitochondrial genetic

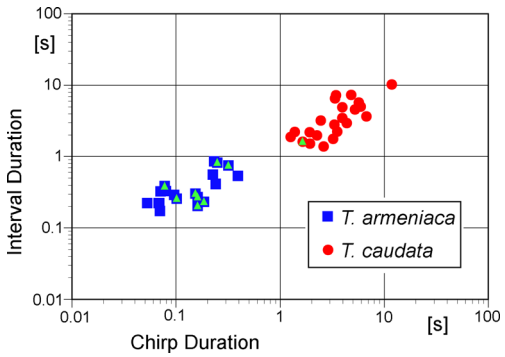

Fig. 7 Relationship between the duration of chirps and inter-chirp intervals in *T. caudata* and the *Tettigonia armeniaca* complex. *Green triangles* mark recordings from Ispir, Turkey, where monosyllabic, disyllabic, and polysyllabic songs of *T. armeniaca* were recorded, as well as a song of *T. caudata* (Color figure online)

differentiation found in this study suggests relatively recent diversification and fast expansion throughout the Palaearctic and the occasional presence of hybrids, even between members of different clades (Schul 1995), are in support of the latter suggestion. The occurrence of the most basal branching resulting in the topology clade C (*T. cantans*) + (clades A, B) supports the ancestral state of the temporal song structure and the low female preference filter of *T. cantans* (Schul 1998). Furthermore, this type of song (although female acoustic preference is not known) occurs in *T. uvarovi*, the basal taxon in clade B. Interestingly, *T. uvarovi* has relatively short wings but is similar in overall habitus to *T. viridissima*, which is another piece of evidence suggesting a closer relationship between clades A and B. While both *T. cantans* and *T. uvarovi* are typically found in humid habitats with a moderate to cool climate and occupy the northernmost areas of the range of this genus, *T. caudata* and the *T. armeniaca* complex occur mostly in mountainous and steppe areas from southeastern Europe, through Anatolia and Iran, to Central Asia. Thus, song elaboration by changes in syllable length and the development of echemes may have been connected with the southwestern expansion from the Eastern Palaearctic towards drier open habitats in the mountains of Central Asia and/or Irano-Anatolia.

The uniform morphology, intraindividual and intrapopulation song variability, and low genetic distances suggest that all studied members of the *T. armeniaca* complex represent a single taxonomic unit. The variable song pattern within the complex is a unique phenomenon in bush-crickets. The incorporation of either long monosyllables or short disyllabic or polysyllabic echemes in communication indicates an unusual mechanism of song recognition. The recognition mechanism of females of the related *T. caudata* requires mainly echemes that do not contain long intervals, i.e., echemes composed of a fast sequence of syllables (high syllable repetition rate; Schul 1998). Those females accept even continuous songs without intervals (Schul 1998). Yet, the minimum acceptable duration of an echeme has not been tested. Females of the *T. armeniaca* complex may use the same criterion, possibly accepting shorter echeme durations than *T. caudata* and rejecting longer ones. In this case, the internal structure of a chirp can vary as long as there are no long intervals within a chirp. Populations of the *T. armeniaca* complex occur sympatrically with *T. caudata* (their suspected syntopic occurrence may be marginal or accidental), and thus, low selectivity could provoke hybridization. Yet, we found neither significant differences in the song frequency (unpublished data) and duty cycle (Fig. 6), nor evidence for hybrids between these groups. Thus, to elucidate the mechanisms of recognition and sexual selection in this group, it is necessary to conduct large-scale population genetic research combined with a behavioral study of female acoustic preferences.

Clade A is composed of a group of populations with relatively uniform song patterns, more or less typical of

T. viridissima. In contrast to the *T. armeniaca* complex discussed previously, here, a uniform song pattern was present within a rather wide range of morphotypes. However, variation concerned only body size and wing length, while the shape of the female external genitalia and the male internal and external genitalia did not differ across the morphotypes. Although the song pattern showed moderate geographical structuring (shorter echemes in North African populations), genetic data revealed a mixed pattern of all sampled populations. The duty cycle distribution (Fig. 6) largely overlapped between Eurasian and North African samples. The previous data suggest a recent expansion of *T. viridissima* populations to the African continent (possibly during the glacial maxima connected with the ocean level decreasing since the Pleistocene). Thus, isolated micropopulations may have specialized in certain microclimates prior to subsequent secondary contact and gene exchange. Similar morphological changes involving shorter wings have been observed in *T. viridissima* populations occurring in Great Britain (Cooper et al. 2012).

Taxonomic reconsiderations

Following the interdisciplinary study presented previously, we suggest the following taxonomic reconsiderations:

T. viridissima (Linnaeus, 1758)

T. vaucheriana Pictet, 1888, syn.n.

T. maroccana Bolívar, 1893, syn.n. (upon synonymy with *T. vaucheriana*)

T. lozanoi (Bolívar, 1914), syn.n.

T. longealata Chopard, 1937, syn.n.

T. krugeri Massa, 1998, syn.n.

Notes, diagnosis, and distribution In Northern Africa, two morphological groups of species occur. The *Tettigonia savignyi* group contains *T. savignyi* (Lucas, 1849) and *Tettigonia macroxipha* (Bolívar, 1914) (including also *Tettigonia longispina* Ingrisch, 1983, described from Sardinia) that characterizes with male cercus with a very big internal spine (longer than the width of cercus), titillators with short stout apical arms with a very small second apical hook and apically attenuated female subgenital plate with a narrow incision. This group has not been considered in the present study.

The second group involves species related to *T. viridissima* and here assigned to as *T. vaucheriana* complex (among the listed in Eades et al. 2016, valid taxa are *T. vaucheriana*, *T. lozanoi*, *T. longealata*, and *T. krugeri*). The latter characterizes with male cercus with a short internal spine (much shorter than the width of cercus), titillators with long gracile apical arms with two equal apical hooks, and apically widened female subgenital plate with a wide incision. Formerly, the latter taxa were considered distinct species on account of differences in the length of tegmina, body size, and width of scapus in relation to the first antennal segment and subtle differences in the female subgenital plate (based on dry specimens in which its shape may differ after deformation due to desiccation). With the present study, we found all these characters highly variable between and within populations. For example, width of scapus is in direct relation to the body size (the stouter the body is, the wider the scapus is in relation to the first antennal segment). Considering the low genetic distances, uniform male and female genitalia, and the uniform song of the studied populations subjectively referred to at least three of the mentioned taxa, we consider all members of the *T. vaucheriana* complex synonymous with *T. viridissima*. Thus, the species distribution, known to be mostly restricted north of the Mediterranean, is now proved to cover all Western Palaearctic including the southern Mediterranean on the territories of northern Morocco, Algeria, and partly Libya (thus, without any doubt, also Tunisia).

T. armeniaca Tarbinsky, 1940, stat. nov.

T. acutipennis Ebner, 1946, syn.n.

T. turcica Ramme, 1951, syn.n.

Notes, diagnosis, and distribution Two species related to *T. caudata* and usually recognized by their shorter wings are known to occur in Anatolia (*T. acutipennis* and *T. turcica*) (Ebner 1946; Ramme 1951). Similarly to *T. caudata*, these taxa are characterized by well-visible black dots in the base of the ventral spines of the femora (most visible in the apical half of hind femora), strongly apically attenuated stridulatory file, long ovipositor (as long as or longer than body, while shorter in most other *Tettigonia*), short and apically outcurved male cerci, and male titillators with comparatively stout apical arms ending with two short hooks. *T. acutipennis* and *T. turcica* differ from *T. caudata* by the shorter (usually less than 33 mm, while over 33 in *T. caudata*) and apically tapering tegmina, shorter hind femora (usually less than 25 mm, while over 25 in *T. caudata*), and less expressed black dots ventrally on the femora. From the Caucasus area, the subspecies *T. caudata armeniaca* has been described by Tarbinsky (1940) and later synonymized with *T. caudata caudata* by Stolyarov (1983). Our samples showed that the morphotype of *T. armeniaca* complex is widely distributed in the Transcaucasus area. Summarizing the results of the current study, we prove that *T. armeniaca* complex represents a single well-outlined species, *T. armeniaca*, stat. nov., with two newly established synonyms, *T. acutipennis*, syn.n., and *T. turcica*, syn.n. *T. armeniaca* characterizes with the previously mentioned features, as well as by its unique variable song and low genetic distances between its populations. Acoustically, it differs from *T. caudata* by the shorter chirps (echemes) and chirp intervals, both being less than a second, while over 1 s in *T. caudata*.

T. armeniaca occurs in moderately humid grass and shrub associations in the high plateaus and mountains of Eastern

Anatolia and whole Transcaucasia (Georgia, Armenia, and Azerbaijan), as well as in the Northern Iran (at least in the Elburs range) (this study and own unpublished data). Its occurrence further east in the mountains of Central Asia is not excluded.

Conclusions

The taxonomy of *Tettigonia* was hitherto based only on morphological descriptions that frequently led to difficulties in outlining its systematics and relationships between taxa (see references in the "Introduction" section). In the present study, we partially revealed the phylogeny and relationships of the genus *Tettigonia*, with a focus on the major groups in the Continental Palaearctic. Three main lineages were outlined representing three distinct clades with unique morpho-acoustic evolution. The combination of variable morphology and uniform song in the *T. viridissima* lineage, and of variable song and uniform morphology in the *T. caudata/armeniaca* lineage, addressed a multitude of evolutionary and behavioral questions. This paper provides a foundation for future investigations into the evolution of the recognition mechanisms and female choice in *Tettigonia*, which led to this diversity of forms, being the cause or result of the ecological success of Green Bush-crickets.

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